



**IMPACT OF AIR POLLUTION ON  
ROOT-NODULATION AND POWDERY MILDEWS  
ON SOME SELECTED PULSE CROPS**

**DISSERTATION**

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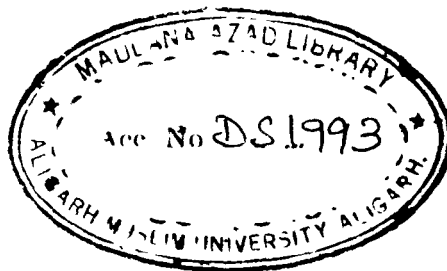
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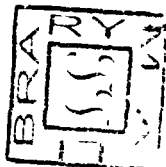
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She is allowed to submit this dissertation for  
consideration of award of degree of MASTER OF PHILOSOPHY  
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## INTRODUCTION

An undesirable change in the physical, chemical and biological characteristics of air, land and water, that may or will harmfully affect human and plant life directly or indirectly, or that of desirable species, industrial process, living conditions and cultural assests or that may or will waste our raw materials resources is called pollution (Odum, 1971). Natural resources are being exploited continuously by man for improving the quality of human life. Consequently, the production of wastes is increasing year by year. The rapid increase in human population and increasing demands for better life are causing ever increasing stress on natural resources. The resulting effects are rapid industrialization and urbanization and increased transportation of industrial products. By-products of industries and transportation are being added continuously to the environment and their amount will gradually increase. Industrial effluents are released in air, land and water.

The toxic substances, responsible for pollution are referred to as pollutants . Pollutants causing air pollution are air pollutants. These can be divided into two basic categories, i.e. gaseous and particulate. The important gaseous air pollutants are sulphur dioxide ( $\text{SO}_2$ ), oxides of nitrogen ( $\text{NO}_x$ ), carbonmonoxide ( $\text{CO}$ ), ammonia ( $\text{NH}_3$ ), chlorine ( $\text{Cl}_2$ ), ethylene ( $\text{C}_2\text{H}_6$ ), hydrogen fluoride ( $\text{HF}$ ), ozone ( $\text{O}_3$ ), peroxyacetyl nitrate (PAN) etc.

The particulate air pollutants are coal dust, flyash, cement dust, and soil dust particles. Wood (1968), on the basis of their origin, divided the air pollutants into two kinds.

- (1) Primary air pollutants which originate directly from the source, e.g.  $\text{SO}_2$ ,  $\text{CO}$ ,  $\text{NO}_x$ ,  $\text{NH}_3$ ,  $\text{HF}$  etc.
- (2) Secondary air pollutants which are produced by reaction of primary pollutants with other environmental factors, e.g.  $\text{O}_3$ , PAN, etc.

These pollutants affect the living organisms by direct contact or with the atmospheric precipitation. 'Acid rain' is mainly caused by  $\text{SO}_2$ , where  $\text{SO}_2$  reacts with water to form sulphuric acid. Sulphuric acid falls with precipitation in the form of 'acid rain' (Oden, 1968).

Certain ranges of environmental factors are necessary for the proper and healthy growth of the plants. The quality of air is very important for plant health, as over 90% biomass of green plants is derived from atmosphere. Plant growth and yield are adversely affected, directly or indirectly by air pollution (Mudd and Kozlowski, 1975; Heck et al., 1982). Air pollutants induce injuries of various kinds in a number of agricultural and horticultural crops. The Environmental Protection Agency (EPA), in U.S.A. estimated that in 1976 annual losses to agriculture production caused by poor air quality was around 2.9 billion



dollars. Yield losses have been found to be caused by air pollution in various crop plants including soybean, peanut, cotton, tobacco, vegetable crops, ornamentals etc.

Direct injury to leaf tissue or interference in biochemical reactions in leaves are main effects of gaseous air pollutants after their entry through stomata (Pell, 1979). Particulate air pollutants fall and deposit on the leaf surface forming a thin encrustation on the leaf surface. Transpiration is influenced and the transmission of solar radiation is affected (Darley, 1966). The sulphuric acid or nitric acid either directly injure the plant parts or indirectly through soil, harm the root system. Air pollutants affecting physiology and biochemistry of the plants, induce visible symptoms like chlorosis, necrosis, early senescence, stunting etc (Heagle, 1973; 1982; Agrios, 1988).

Powdery mildew fungi belong to order Erysiphales and family Erysiphaceae. They are obligate parasites of angiosperms, often showing a great physiological specialization, having races confined to a narrow range of host plants. The genera which are more important than others

are Sphaerotheca, Erysiphe, Podosphaera, Microsphaera, Uncinula, Leveillula, Phyllactinia. All parts of plants except roots are infected by powdery mildews. They infect primarily leaves but stems, buds, flowers and fruits may also be infected in case of severe attack. Plant parts involved in infection depends upon the host plant and powdery mildew species. Some of their other characteristics are the occurrence of haustoria in the epidermal cells of their hosts, rapid development in rain free seasons and higher water contents of their large turgid, airborne conidia.

Powdery mildews fungi are one of the most important group of plant pathogenic fungi, which infect a large number of plants of economic importance across the world. Abundant conidial development by powdery mildew fungi as a superficial growth on the host surface, mainly foliage, gives powdery appearance of the infected plant parts. Black minute fruiting bodies may also develop on the infected parts of plants. There are some reports which suggest that air pollutants like  $\text{SO}_2$  and  $\text{O}_3$  inhibit powdery mildew on some plants and reduce the disease severity. Oak trees were found to be free from powdery mildew caused

by Microsphaera alni near a paper mill in Austria (Kock, 1935). It was shown later by Hibben and Walker (1966) that exposure of conidia of M. alni to  $\text{SO}_2$  at 0.30 ppm to 0.50 ppm for 72 h, resulted in their decreased germination and disease development, did not proceed beyond appressorium stage. The mature conidia of Erysiphe graminis infecting barley leaves when exposed to 0.10, 0.20 or 0.30 ppm of  $\text{O}_3$  for 24 h during incubation, appeared to be in critical stage and the infection was reduced due to sensitivity of the fungus to  $\text{O}_3$  (Schuette, 1971).

The organisms, parasitic or non-parasitic associated with plants growing under air pollutants stress are likely to be affected directly or indirectly. Symbiotic nitrogen fixation by rhizobia in leguminous crops is a very significant natural biological process and important for nitrogen economy of soil. Air pollutants affect nitrogen fixation and root-nodulation. The inhibition of  $\text{N}_2$ -fixation results from reduction in the nodulation and suppression in bacterial population. These effects are caused by the impact of air pollutants on plants. Ozone ( $\text{O}_3$ ),  $\text{SO}_2$ , and particulate matters are reported to suppress N-fixation by the species of Rhizobium (Tingey and Blum, 1973; Shriner and Johnston, 1981).

Some workers have observed reduced root nodulation, nitrogen fixation, and/or leghaemoglobin content of leguminous plants after following 1 or 2 acute  $O_3$  exposures in greenhouse or controlled environment chambers (Blum and Heck, 1980; Blum and Tingey, 1977). Root nodulation by Rhizobium in kidney beans grown in greenhouse or outdoor plots and soybeans grown in greenhouse (Shriner, 1970; Waldron, 1978) was reduced by sulphuric acid rain of pH 3.2.

Air pollution impacts are now becoming increasingly evident in India. Rapid industrialization and urban growth are causing danger to air quality. Thermal power plants, oils refineries, and other big and small industries using coal or oil as fuel are major sources of air pollution in India. Agricultural crops grown around such industries, might suffer significant yield losses caused by air pollutants. Additionally, if one or other disease becomes more aggressive under specific pollutant conditions, the crop may be greatly damaged. The foliar diseases like powdery mildew may be suppressed. Similarly suppression of root nodulation on legumes may be caused by such air pollutants. Although there are some studies on impact of air pollution on legumes, powdery mildews (Heagle, 1973, 1982) or root

nodule bacteria (Shriner, 1974; Waldron, 1978), no attempt has been made to examine effect of air pollutants on powdery mildews and root nodulation and its cumulative effect on plant growth and yield of legumes. Currently there is emphasis for increasing the productivity of pulses in our country. Pulses are major source of protein for the vast vegetarian population of the country. Therefore, in the proposed study for Ph.D., experiments will be conducted to determine the effects of air pollutants ( $\text{SO}_2$ ,  $\text{O}_3$ ) on powdery mildews and root nodulation on two pulse crops (pea and blackgram) and their cumulative impact on growth and yield.

It is expected that this system of three living components, host (pulses), parasite (powdery mildew) and nodule-forming bacterium (Rhizobium sp.) under air pollution stress will be suitable to approximate the field conditions. To quantify the effects of various kinds of air pollutants, experiments will be conducted under glasshouse conditions using air pollutant exposure chambers.

## LITERATURE REVIEW

Air pollution has now entered as a new factor in agriculture and crop damages caused by air pollutants are now recognised in different parts of the world. Several chemical substances present in the air surrounding various kinds of industries are toxic to plants. The four most important air pollutants in the order of their phytotoxicity are ozone ( $O_3$ ), sulphur-dioxide ( $SO_2$ ), nitrogen dioxide ( $NO_2$ ) and ammonia ( $NH_3$ ). Ozone alone or in combination with  $SO_2$  and/or  $NO_2$  causes crop losses upto 90% in some cases (Heck et al., 1982). Consequently, these phytotoxic air pollutants are of great concern to agricultural scientists. Air pollutants injure plant foilage, significantly alter their growth and yield, and change the quality of the marketable plant products. The air pollutants also increase or decrease plant diseases caused by biotic pathogens (Heagle, 1973, 1982). Air pollution is analogous in many ways to plant diseases (Pell, 1979).

On the basis of their physical appearance, air pollutants are grouped into two categories-gaseous and particulate (Wood, 1968).  $O_3$ , PAN,  $SO_2$ ,  $Cl_2$ ,  $C_6H_6$ , HF,

$H_2S$ ,  $NO_x$  etc. are common gaseous air pollutants. Coal dust, fly ash, cement dust, soil dust particles etc. are major particulate air pollutants. Primary pollutants like  $NO_x$ ,  $SO_2$ , when come in contact with the water in atmosphere and atmospheric precipitation, are converted into acids and fall down. This condition of environmental pollution is called 'acid rain' (Liken and Barman, 1974). Acid rain is supposed to be the most acute and severe air pollution problem in developed countries, while the particulate air pollutants are major problem in developing countries (Das, 1986). In India, 40-44% of air pollutants are particulate matters and are a threat to plants and other living organisms (Das, 1986).

#### Air pollution effects on plants

Studies on plant diseases caused by air pollutants began in 19th century (Heagle, 1973). The extent and nature of injury or damage caused by air pollutants is determined by genetic and environmental factors of plant, as well as by level and duration of exposure to pollutants (Heagle, 1973). Air pollutants affecting physiology and biochemistry of plants induce visible symptoms like chlorosis, necrosis,

early senescence, stunting and several other symptoms depending upon types of air pollutants and plants involved (Darley and Middleton, 1966; Brandt and Heck, 1968; Barret and Benedict, 1970). The existing information on some aspects of effects of air pollutants on plants are briefly reviewed herewith.

### Sulphur dioxide ( $\text{SO}_2$ )

Sulphur dioxide is one of the most important air pollutants that cause damage to plants. It is emitted to atmosphere through combustion of coal and coal products, refining and utilization of petroleum and natural gas manufacturing and industrial utilization of sulphuric acid and sulphur and the smelting and refining of ores, especially of copper, zinc, lead and nickel. The combustion of coal is a major source of atmospheric  $\text{SO}_2$ . The amount of  $\text{SO}_2$  emitted from coal depends upon the sulphur content of the coal which varies from 1-6% of total weight. Coal-burning power plants represent the most important single source of  $\text{SO}_2$  (Wood, 1968). The concentrations of  $\text{SO}_2$  at ground levels depends upon the amount and concentrations of emission, distance from the source and meteorological and topographical



conditions. In general  $\text{SO}_2$  concentration decreases rapidly with distance from the source and with increased air movement.  $\text{SO}_2$  concentration near point sources, such as coal burning power plants and smelters, with little or no pollution control equipment, may be as high as 1 to 3 ppm.  $\text{SO}_2$  concentration in large urban areas may range from 0.05 to 0.40 ppm (Heagle, 1973, 1982).

The mode of entry of  $\text{SO}_2$  in plants and the overall mechanism of damage of leaf tissue have been examined by various workers.  $\text{SO}_2$  enters the leaves through stomata and reacts with water in mesophyll tissue to produce sulphite ion, which is slowly oxidised to sulphate ion. The sulphate ion may be utilized by the plant as a nutritional sulphur and converted to organic form (Thomas et al., 1944). In excess amounts sulphite and sulphate ions become toxic to plant cells. Sulphite ions are much more toxic than sulphate ions (Thomas et al., 1943).

$\text{SO}_2$  causes two general types of symptoms or injuries referred to as chronic and acute. Accumulation of sulphite ions causes appearance of both of these symptoms. General chlorotic appearance of the leaf, mild chlorosis, yellowing of leaf, silvering or bronzing of the under surface of the

leaves are considered as chronic symptoms. Some plants show white types of chronic markings and red, brown, or black patches on the leaves (Barrett and Benedict, 1970). Absorption of lethal quantities of  $\text{SO}_2$  leads to acute symptoms on plants. Tissues of marginal or inter-costal areas of leaves become dead. First they become greyish green water-soaked in appearance but later on drying become bleached ivory in colour. The dead or necrotic areas may fall out and the leaves give a very ragged appearance. When major portion of the leaf is injured, it is shed by formation of an abscission layer at the base of the petiole (Barret and Benedict, 1970). At low concentration,  $\text{SO}_2$  causes chlorosis of leaves without formation of necrotic lesions and the veins characteristically remain green (Darley and Middleton, 1966; Agrios, 1978, 1988).

Sulphur dioxide affects both physiological and biochemical processes of plants. Photosynthesis of affected plants is generally reduced, but transpiration and dark respiration are increased. Such effects have been observed both in short and long term exposures of plants (Black and Unsworth, 1979; McLaughlin et al., 1979a; Takemoto and

Noble, 1982; Saxe, 1983).  $\text{SO}_2$  effects on enzyme systems and metabolic processes are related to  $\text{SO}_2$  concentration, plant species, plant age and environment. In some cases, enzyme activity is increased by exposure of the plants to low level of  $\text{SO}_2$  and decreased by higher concentration (Horsman and Wellburn, 1977; Soldatini and Ziegler, 1979; Wyss and Brunold, 1980; Piere and Quieroz, 1982; Tanak et al., 1982).  $\text{SO}_2$  affects plant metabolism in a variety of ways. It stimulates phosphorus metabolism (Plesnicar, 1983) and reduces foliar chlorophyll concentration (Pandey and Rao, 1978; Lauernorth and Dodd, 1981). Carbohydrate levels are increased by low and decreased by higher concentrations of  $\text{SO}_2$  (Kozoil and Jordan, 1978).

Adverse effects of  $\text{SO}_2$  on physiology and biochemistry of plants significantly influences their growth, development and productivity. These effects of  $\text{SO}_2$  on crop plants have been shown in studies conducted both in glasshouse and ambient conditions. Pandey and Rao (1978) exposing wheat plant to  $\text{SO}_2$  recorded reduction in root and shoot lengths, number and area of leaves per plant, biomass, productivity and number of grains per spike. No chlorosis or necrosis

of the leaves were, however, observed. Sprugel et al. (1980) exposed soybean to 0.09 to 0.79 ppm in an open air fumigation chamber and found significant reduction in yield. Leaf injury was not frequently observed. Slight increase in biomass was, however, reported in alfalfa exposed continuously to 0.036 ppm concentration of  $\text{SO}_2$  (Lockyer and Cowling, 1981). Exposure of tomato plants to 0.12 ppm concentration of  $\text{SO}_2$  for 72 h/week for 5 or 10 week, caused no effects on fruit yield and other soluble and total solid contents but slight decrease in ascorbic acid of ripe fruits occurred (Lotstein et al., 1983). In Vigna sinensis exposed to 0.12, 0.25 and 0.5 ppm of  $\text{SO}_2$ , bifacial necrotic lesions appeared on the middle and lower leaves exposed to 0.25 and 0.5 ppm  $\text{SO}_2$ . A slight stimulation in plant height, root and shoot lengths was observed in 0.12 ppm  $\text{SO}_2$  exposure in early stages of plant growth (Kumar and Singh, 1986). Exposure of intact pinto beans and those with cotyledons removed immediately after germination to 0.15, 0.25 and 0.50 ml/lit.  $\text{SO}_2$  at an identical dose (0.50 ml/lit/h/day) for 4 weeks reduced leaf area, shoot and root dry weight and increased shoot/root ratio and specific leaf areas in all exposed plants (Temple et al., 1985). Physiological measurements, injury rating

and collection of leaf material for chemical analysis of winter wheat plants exposed to  $\text{SO}_2$  in open top chambers were carried out after 22 days of exposure. Higher  $\text{SO}_2$  concentration caused decrease of buffering capacity, increase of total sulphur content and increase of injury of wheat levels (Bytnerowicz et al., 1987). Broad-bean crops (Vicia faba L.) exposed to elevated  $\text{SO}_2$  concentrations in an open-air field exposure system for the controlled released air pollutants at  $165 \text{ ug/m}^3$ ,  $62 \text{ ug/m}^3$ ,  $74 \text{ ug/m}^3$ , showed strongly affected leaf area development during the pod-filling. Sulphur content was strongly high in the leaves and pods of the fumigated plants and the Ca content of the leaves was reduced by  $\text{SO}_2$ , chlorophyll content of different leaf was unaffected by  $\text{SO}_2$  (Kropff et al., 1989). When the seeds of chick pea and lentil were exposed to  $\text{SO}_2$  at 0.1 ppm and 0.2 ppm concentrations respectively, seed germination was found to be inhibited and the post-emergence mortality of the seedlings occurred. Chick pea was more sensitive than lentil to the exposures at germination stage. But after emergence the seedlings of lentil exhibited more sensitivity than chick pea. When plants were exposed to

SO<sub>2</sub> at both the concentrations, reduction in plant growth, yield, leaf pigment, seed protein, number of stomata, number and length of trichome hydadothes occurred (Singh, 1989). Chlorosis and small dot like patches, yellow to grey in colour appeared on the tomato leaves exposed to SO<sub>2</sub> 0.1 and 0.2 ppm respectively. There was reduction in number and size of stomata, carotenoid content, chlorophyll, yield, plant growth, number of fruits/plant (Khan, 1989).

#### Oxides of nitrogen (NO<sub>x</sub>)

Nitric oxide (NO) and nitrogen dioxide (NO<sub>2</sub>) are the two important gases in the nitrogen oxide group of air pollutants. They are produced primarily by high temperature combustion (Taylor and MacLean, 1970). Nitrogen oxides are produced from the oxygen and nitrogen in the air by hot combustion sources, such as open fires, furnaces and automobile combustion chambers. Combustion of petroleum products are the major source of NO<sub>x</sub>. In this process nitric oxide is oxidised to nitrogen dioxide (Benedict and Breen, 1955b, Agrios, 1978, 1988). Nitrogen dioxide in concentrations of 2-3 ppm causes bleaching of plants, necrotic lesions and excessive defoliation (MacLean et al., 1968;

Agrios, 1978, 1988). There is no report of visible symptoms of leaf injury from nitric oxide (Taylor and MacLean, 1970). Acute foliar markings are produced by high concentrations. Water-soaked lesions, first appear on the upper leaf surface followed by rapid tissue collapse. These lesions with time extended throughout the leaf and produce small irregular necrotic patches. The necrotic patches are usually white to tan or brown, but some time bronze in colour. The interveinal lesions are prominent at the apex and along with margins, but may occur on the leaf surface (Benedict and Breen, 1955b; Middleton et al., 1958; Taylor and Eaton, 1966; MacLean et al., 1968).

The expected severity of injury to plants depends upon  $\text{NO}_2$  concentration and duration of exposure. However, in the case of high concentration exposures, there is no direct relationship between time and concentration, except within very narrow ranges. The concentration of  $\text{NO}_2$  is more important than the exposure duration in determining the extent of injury (MacLean et al., 1968). Rogers et al. (1979) in the study using ambient levels (0.097-0.152 or 0.325 ppm) of the  $\text{NO}_2$  for 3 h, found that all the nitrogen was metabolized in the plant system and showed no significant

effect on beans. There are evidences that N derived from  $\text{NO}_2$  is absorbed by the exposed plants and translocated throughout the plant tissues and metabolized into amino acids, proteins, nucleic acids and some lipids (Matsumaru et al., 1979; Rogers et al., 1979; Yoneyama et al., 1980).

Reduction in the tuber number and weight and in the total yield was found when potato plants were exposed to high dosage of  $\text{NO}_2$  (Sinn and Pell , 1984). Exposure of two-day-old plants of spring barley to 100 ml/lit.  $\text{NO}_2$  for 20 days, resulted in a very little effect on growth (Pande and Mansfield, 1985). No lesions developed on Cicer arietinum plants exposed to  $\text{NO}_2$ , but the leaves became dark green and significant reductions in dry weight fraction of both root and shoot occurred (Kumar and Singh, 1985). In soybean, the photosynthetic rate was increased by 18 and 23% immediately, following exposure to 0.2  $\mu\text{L/L}$   $\text{NO}_2$ , but reduction in the photosynthetic rate by 23 and 50% occurred immediately after exposure to 0.5  $\mu\text{L/L}$  (Sabaratnam et al., 1988). Godzik et al. (1985) exposed the radish cultivars to  $\text{NO}_2$  at a concentration of 950  $\mu\text{g/m}^3$  for 3 h, and recorded an increase in the amount of leaf injury.



## Fluoride

Fluoride is a natural components of soil, rocks and minerals (MacIntire, 1945). When these materials are heated to high temperature or treated with acid during industrial processing, toxic quantities of fluoride may be released into the atmosphere. The industries which produce aluminium, steel, ceramics, phosphorus, chemicals, fertilizers etc. are the major sources of fluoride (Treshow and Pack, 1970; Heagle, 1973). The major fluorides which are injurious to the plants are silicon tetra fluoride ( $\text{SiF}_4$ ) and hydrogen fluoride (HF). Hydrogen fluoride is generally considered to be most important (Heagle, 1973).

Gradual accumulation of fluoride in the plant tissue over a period of time causes injury to plants. The exposure duration and atmospheric concentration are two important determinants of the severity of injury. The expression of symptom, however, is not always related strictly to the fluoride dosage because appreciable amounts are often washed off from plant foilage by rain and in some cases fluoride may be converted within the tissue into the forms that are not injurious. Generally plants take up the small amount of

fluoride from the soil, and plant injury is negligible even when the soil fluoride content is relatively high (Haselhoff et al., 1932; Mac Intire et al., 1942).

Adsorbed fluoride is rapidly translocated to leaf tips and margins (Jacobson et al., 1966). Fluoride enters the plant leaves through the stomata, passes into the intercellular spaces and is absorbed by the mesophyll from where it may move to other cells (Thomas and Hendricks, 1956). With the transpiration stream fluoride moves to the leaf tips and margins for accumulation, where the concentration increases several times higher than the average concentration in the leaf as a whole (Zimmerman and Hitchcock, 1956).

The fluoride causes symptoms on leaves, flowers and fruits. Chlorosis and necrosis of leaves may occur. Lesions may be grey or light green at first but later become reddish brown to tan. Abscission of leaves is also induced by fluoride exposure (Heggestad 1968). Necrosis in the petals and sepals of flowers and premature ripening of fruits are also caused by fluoride (Treshow and Pack, 1970). Jacobson et al. (1966) observed no injury in cotton even though leaves contained 4000 ppm of the fluoride. Nevertheless, injury in plants particularly in leaves occurs most

commonly after several weeks of exposure to fluoride, though injury may appear as a necrotic and chlorotic lesions. With continued exposure, in a few hours at high concentrations lesions enlarge and entire leaves may become necrotic. Grapefruits develop a distinctive chlorotic pattern after prolonged exposure to fluoride (Heggsted, 1968).

When soybean, maize, peanut and navy bean (Phaseolus vulgaris) were exposed to HF at concentration  $0.26 \text{ ug/m}^3$  or  $< 0.03 \text{ ug/m}^3$  separately for 8 h /day, in an open top chamber from the seedling stage to harvest, the gas caused little effect on soybean; increased the dry weight of maize cobs; reduced the weight of kernels per plant of peanut; and increased the number of pods in bean per plant, but average bean weight was reduced in navy bean (Phaseolus vulgaris) (Murray and Wilson, 1990).

#### Ammonia

Anhydrous ammonia used as a fertilizer in the field is reported to cause the field injury of plants. Ammonia causes acute tissue collapse in the leaves with or without subsequent loss of chlorophyll. Leaves show a cocked green

appearance, becoming brown or remaining green on drying. Benedict and Breen (1955a; 1955b) in a field observation recorded extensive and widespread injury of plants within 1-2 miles of the ammonia spill. Complete collapse of leaf tissue occurred close to the sources. Several sensitive species showed blackened tissue at a distance upto a mile from the spill. In necrotic areas bright tan colour developed. Several cereals and grasses showed necrotic and chlorotic interveinal streaking at some distance from the spill. According to Thorntan and Setterstorm (1940) buck wheat, coleus, sunflower and tomato at 40 ppm exposure of ammonia for 1 h caused significant injury, but a slight marginal injury when exposed at 16.6 ppm for 4 h. When tomato plants were exposed to 0.1 and 0.2 ppm. At 0.1 ppm  $\text{NH}_3$  exposure plant showed no symptoms while at 0.2 ppm exposure produced browning of leaf margins after more than a month of start of exposures. Significant reduction in length and fresh and dry weights of shoots and roots, number of flowers/plant, carotenoid, chlorophyll a, number of trichomes and stomatal size and aperture occurred (Khan, 1989).

## Ozone ( $O_3$ )

Ozone is most important air pollutant of photochemical oxidant. Automobile exhausts and other internal combustion engines are important sources of ozone. Incompletely burned hydrocarbons and  $NO_2$  are released into the atmosphere by the automobile exhaust. In the presence of UV light, this  $NO_2$  react with oxygen and forms  $O_3$  and NO. The ozone may react with NO to form the original compound. But in the presence of unburned hydrocarbons the NO reacts with hydrocarbons instead of  $O_3$  and  $O_3$  is released in the atmosphere (Agrios, 1978, 1988).  $O_3$  concentration at ground level is generally less than 0.03 ppm.

Ozone is injurious to plant leaves exposed for even a few hours at concentration of 0.1 to 0.5 ppm. Ozone enters leaves through stomata and causes injury to palisade parenchyma and other cells of leaves by disrupting the cell membrane. Affected cells near stomata collapse and die and white (bleached) necrotic flecks appear, first on the upper side and later on both the leaf surfaces. The colour of the affected leaves varies from light tan to red or almost black, depending upon the plant involved. Affected leaves of some

plants such as citrus, grapes and pines fall prematurely (Darley and Middleton, 1966; Agrios, 1978, 1988). Epidermal cells remain uninjured while the palisade cells and spongy mesophyll become injured. Many injured cells remain alive but chloroplast is disrupted and the chlorophyll amount is reduced significantly (Hill et al., 1961).

Ozone is reported to cause various types of damages in a number of economically important crops such as soybean, potato, cotton, pepper, sunflower, clover etc. Exposure of seedlings of sensitive tomato cultivars to 0.4 ppm of  $O_3$  for 2 h, repeated for 6 time caused 57% reduction in growth, when treated seedlings were transplanted in field (Henderson and Reinert, 1979). Pell et al. (1980) observed a decrease in tuber weight and total solids, but increase in reducing sugars in potato, exposed at several growth stages to 0.2 ppm  $O_3$  for 3 h in glasshouse. Blum et al. (1983) recorded 14 and 27% reduction in clover regrowth during second year at 0.06 ppm and 0.09 ppm  $O_3$ , when exposed to 0.03, 0.06 and 0.09 ppm  $O_3$  for 7 h/day in the field conditions for 2 years. Exposure of soybean to 0.022 ppm and 0.112 ppm  $O_3$  for 7 h/day in the field, caused a reduction in yield and oil

content by 39 and 126% respectively, while protein content was not affected (Greenwald and Endress, 1984). Heggestad et al. (1985) showed a 5% reduction in the yield by exposing soybean plants to  $O_3$  in open top chambers. Kats et al. (1986) exposed 3 cultivars of rice, M7, M9 and S201 in open top chambers to ozone and recorded a reduction in seed size and seed sterility in those plants which produced more panicles. Heggestad et al. (1987) exposed tomato cultivars to 0.011, 0.059, 0.118, 0.235 ppm and 0.468 ppm in open field chambers with non-filtered (NF) air and 0.005, 0.113 and 0.466 ppm with charcoal-filtered (CF) air for 57 days at 5 h/day and 5 days/week. A decrease in ripe fruit yields by 16% in NF compared with CF air was recorded. Khan (1989) exposed tomato plants to 0.2 ppm  $O_3$  and recorded chlorosis of leaves and reduction in fresh and dry weights of shoot and root and number of fruits per plant. Soybean plants exposed to  $O_3$  showed reduction in seed and growth by 7.9 to 18.6% (King and Nelson, 1987; Heggestad, 1988). Ambient levels of ozone caused little effect on vegetative growth or yields of four field grown tomato cultivars when exposed in open top chamber to charcoal filtered (CF), non-

filtered (NF), but NF plus 1.5 times ambient  $O_3$  concentrations reduced yields from 17% in cv. "UC204C" to 54% for cv. "Hybrid 31" (Temple, 1990). Inhibition of seed germination of chickpea and lentil occurred by exposing pots to two different concentrations of  $O_3$  i.e. 0.1 and 0.2 ppm. There was also reduction in plant growth (length, fresh and dry weights of shoot and root) and number of flowers and fruits per plant at both the levels of  $O_3$  (Singh, 1989).

#### Peroxyacetyl nitrate (PAN)

This secondary air pollutant is produced from the reaction of nitrogen oxide with the unburned hydrocarbons in the presence of U.V. The unburned hydrocarbons and nitrogen oxide released as autoexhaust in the metropolitan cities, result in production of PAN, which create a serious problem (Heggestad, 1968; Agrios, 1978, 1988).

PAN also enters leaves through stomata. Spongy parenchymatous near stomata collapse and are replaced by air pockets causing glazed or silvery appearance of leaves. The symptoms on broad leaved plants appear on the lower leaf surface, while monocot leaves show symptoms on both sides (Agrios, 1988). On exposing to 15-20 ppb of PAN in ambient conditions,



lettuce, Swisschard, pintobbeans, petunia, tomato and African violet showed injury, while corn, onion, begonia and cotton exposed to 75-100 ppb for 2 h to PAN showed no injury (Noble, 1965; Darley et al., 1966; Brandt and Heck, 1968a; Heck et al., 1970). Young leaves were found to be more susceptible to PAN than older leaves. Inhibition of photosynthesis, affecting various aspects such as adenosine triphosphate (ATP) formation, nicotnamide adenine dinucleotide pnosphate (NADP) reduction and CO<sub>2</sub> fixation occurred by PAN (Taylor and MacLean, 1970).

#### Acid Rain

Acid rain is mainly the conversion of SO<sub>2</sub> and NO<sub>x</sub> into acids after coming in contact with water present in atmosphere and with atmospheric precipitation. This acid falls on the ground in the form of rain.

The acidification and alteration of water and soil are primarily the cause of acid rain. Herbaceous plants are more sensitive to direct injury by acid rain than woody plants (Heck et al., 1986). When trifoliate leaves of Phaseolus vulgaris were exposed to simulated acidic rain with high and low pH level, a great difference of leaf and

cell permeability responses to various ions was recorded. A lower adaxial leaf resistance during leaf development was correlated with an increased rate of nutrient leaching at more acidic levels (Lance et al., 1981). In soybean, with increase in acidity reduction in plant height, chaff dry weight, plant population, pods per plant, seeds per plant, seed per pod occurred due to exposure to acidic rain (Porter et al., 1989; Kuja and Dixon, 1989).

Seed germination of chickpea and lentil was suppressed in acidic soil. In relation to germination lentil seeds were more tolerant to acidity of the soil than chickpea seeds. But the seedlings of lentil were more sensitive to acidity and suffered greater mortality after emergence than chickpea seedlings. Simulated acid rains of pH 5 and 3.2 reduced the plant growth, flowers, fruits, chlorophyll contents of leaves, protein contents of seeds, number of stomata, size of stomata and stomatal aperture and number and length of trichome-hydatodes, but increased the number and length of trichomes of chickpea and lentil (Singh, 1989). Tomato plants treated with acidified water at pH 3.2 exhibited bifacial irregular lesions white to tan coloured in inter-veinal areas of leaves and marginal chlorosis. Distortion

of leaf margins also occurred. After 2-3 exposures wilting of leaves occurred (Khan, 1989).

#### Particulate air pollutants

Coal dust, fly ash, lime dust, cement dust and soil dust particles etc. are the major particulate air pollutants. Production of coal, cement, combustion of coal, gasoline, lime kiln operation, soil erosion, agricultural burning and wrong agricultural practices, volcanic eruptions, transportation and construction etc. are the sources of particulate air pollutants. Particulate matters settle on exposed plant parts, mainly foliage. Chlorosis, necrosis and death of the tissues may occur due to heavy deposition of the particles (Darley and Middleton, 1966; Heck et al., 1970). The reduction in quality of vegetative and fruits occur by high particulate emission from the different sources (Heck et al., 1970).

A reduction in transpiration rate, chlorophyll content and productivity of the wheat plants due to cement dust pollution was observed by Singh and Rao (1981). Colwill et al. (1979) observed a poor growth of the plants grown on roadside with highly busy traffic, where particulate matters were

deposited on the leaves. The dusts of varying origin interfere with stomatal functioning mostly by filling and blocking the stomatal aperture (Ricks and Williams, 1974; Fluckiger et al., 1978, 1979). Increase in leaf temperature (Eller, 1977; Fluckiger et al., 1978) and transpiration (Beasley, 1942; Eveling, 1969), reduction in photosynthesis (Darley, 1966) and increase in the uptake of gaseous air pollutants (Ricks and Williams, 1974) occurs. All these effects lead to poor growth of the plants.

Fly ash deposition on soil may also affect plant growth and their productivity. Some recent studies (Khan, 1989; Singh, 1989; Pasha, 1990) showed that plant growth of crops in soil amended with fly ash is greatly influenced. Foliar symptoms like browning of margins and interveinal areas of leaves, with or without chlorosis in tomato (Lycopersicon esculentum), eggplant (Solanum melongena), okra (Ablemoschus esculentus) grown in fly ash amended soil (80% and 90% levels) were recorded. Significant reduction in plant growth, chlorophyll and carotenoid contents of the leaves increase in trichome and decrease in stomata numbers and premature fall of leaves and flowers also occurred (Khan, 1989). There

was gradual increase in all parameters, at 10-25% level of fly ash except in the number of trichomes. At 50% level of fly ash the reduction <sup>occurred</sup>  $\angle$  in all the parameters, but this reduction was less as compared to reduction recorded from 60% level of fly ash onwards.

Fly ash in soil increased the porosity, water holding capacity, conductivity, cation exchange capacity of the soils, organic matter, sulphate and bicarbonate, carbonate contents, but lowered the soil pH. Increasing the level of fly ash i.e. 60% onwards upto 90% resulted in suppression of leaf pigments, seed proteins, stomatal number and size in chickpea and lentil. The number of trichome-hydathodes, however, increased in chickpea (Singh, 1989). Amendment of soil with fly ash (10 and 25%) of Thermal Power Plant origin improved the plant growth, yield and chlorophyll content of leaves of cucumber plants (Pasha, 1990).

### Powdery Mildews

Powdery mildews are one of the most important group of fungi that cause infection to large number of plants of economic importance throughout the world. Powdery mildews belong to a well defined group of Ascomycetous fungi. They produce superficial white mycelium, abundant large colourless or white, non-septate, turgid, air-borne conidia on the host surface (Yarwood, 1978).

Agricultural crops are found to be readily attacked by powdery mildews. The entire plant is covered with white powdery mass affecting not only the yield but also the quality of produce. Legumes are widely grown throughout the world and are found to be attacked by powdery mildew fungi. Yarwood (1954) reported that Erysiphe polygoni infects all parts of bean except pulvini. Leaves infected by E. polygoni showed yellowing or chlorosis (Cook, 1931; Parris, 1941), followed by browning of the infected cells (Shirashi et al., 1976 ), ultimately resulting into their premature fall (Yarwood, 1951).

Yield losses caused by powdery mildews have been

estimated in some crops. Tarr (1955) found 50% losses in the yield of Vicia faba infected by E. polygoni in irrigated crops, north of Khartoum in Sudan. E. polygoni caused losses to the clovers to the tune of 25-40% in Germany (Masurat and Stephan, 1962, 1965), 72-98% to the pea (Iminov, 1965) in Tachkent. Microsphaera diffusa caused 13% losses to seed yield in soybean in Iowa, U.S.A. (Dunleavy, 1980). In India, Uppal et al. (1935) observed that powdery mildew reduced the number of pods of pea plants from 7 to 1 in heavily infected fields, while the infected seeds was approximately 1/3 of the healthy seeds. Taneja and Grover (1980) found that 48 per cent pea plants ~~were~~ infected with E. polygoni.

Physiological changes in powdery mildew infected plants have also been examined by several workers. The rate of transpiration is increased (Ayers, 1976), especially at night (Yarwood, 1947). Heavily infected plants show reduced transpiration (Ayers, 1980). Rate of respiration is increased (Yarwood, 1934a, 1953; Latch and Hanson, 1968), but the rate of photosynthesis is decreased (Kaur and Deshpande, 1980; Gordan and Duniway, 1981). McKeen and Bhattacharya (1969) found decreased cellulose content in the

infected leaves. But the protein (Sturm, 1958), amino acids, flavonoids and phenols (Agrawal et al., 1980) contents are increased. The enzyme such as peroxidase and glucose-5 phosphate dehydrogenase are increased (Stavely and Hanson, 1967), but a decrease in the contents of ascorbic acid and few other amino acids has also been reported (Agrawal et al., 1980).

#### Effect of environment on powdery mildew

A number of environmental factors affect powdery mildew fungi during pathogenesis. Relative humidity and temperature are most important in this respect. The conidia of E. polygoni germinated (Yarwood, 1936b; Kothari and Verma, 1972) at approximately 0% R.H. because of high water content of the conidia i.e. 69% (Jhooty and McKeen, 1965), and of slow rate of water evaporation (Yarwood, 1952). Rupel et al. (1975) recorded greater incidence of E. polygoni on various hosts under dry than in wet conditions. Adverse effect of rains on powdery mildew has been observed (Yarwood, 1934c; 1936a). Hammarlund (1925) also had observed the poor development of powdery mildew during rain. At moderate R.H. there was a greater production of spores than under high R.H. and the spores had more capability to germinate under low R.H. than



under high atmospheric humidity.

Several workers have reported effect of temperature on the germination of powdery mildew conidia and disease development. The optimum temperature for the development of E. polygoni f. sp. pisi on pea was found to be 20°C (Hammerlund, 1925). Soria and Queberal (1973) reported 25.6°C as the optimum temperature of E. polygoni on mungbean. Mignucci et al. (1977) pointed out that day temperature for the development of Microsphaera diffusa on soybean was 18°C.

The minimum, optimum and maximum temperature for the germination of conidia of L. taurica from Cyamospsis psoraloides were 14, 20 to 25°C and 35°C respectively (Kamat and Patel, 1948). Khan (1972) pointed out that conidia of S. fuliginea on cucurbits can germinate at minimum (5°C), optimum (17-20°C) and maximum (25°C) temperature respectively.

Light has also been found to influence the development of powdery mildew (Yarwood, 1934c, 1957). The germination of conidia was, however, unaffected by light (Kothari and Verma, 1972). Yarwood (1934a) suggested that in low light intensities or in darkness photosynthesis was reduced which ultimately adversely affected the supply of carbohydrates to

the fungus. Yarwood (1936a, 1957) further observed that when some portion of petiole of infected bean plants were placed in darkness, the diurnal periodicity of conidiophore maturation of E. polygoni was not affected until after 5 days, while the rest part of the petiole showed diurnal periodicity.

#### Effect of soil fertility and other soil conditions

Some reports show that powdery mildew disease is affected by soil fertility and other soil conditions. Early development of symptoms and high disease incidence were observed in a poorly cultivated soil (Uspenskaya, 1958). Reduced development of powdery mildew was recorded with high nitrogen (Wijngaarden and Bllen, 1969), potassium (Sturm, 1958), phosphorus (Tretyakova and Omelchenko, 1965) and calcium (Thompson and Ferguson, 1976) fertilizers. Higher dose of silicon (Lowig, 1935) and boron (Baton, 1930; Yarwood, 1938) resulted in poor development of powdery mildew on various hosts. Wood (1967) reported that boron deficiency usually led to accumulation of sugars in leaves and powdery mildews on these sugar accumulated leaves (Yarwood, 1934b) developed profusely. Brnould and Van Steyvoort (1962)

observed that magnesium deficiency accelerated the symptoms of powdery mildew fungus.

Brown (1930) observed that high soil pH favoured the development of powdery mildew on cowpea. On the other hand, Yarwood (1931) observed no such effect from a graded series of application of NaOH and  $H_2SO_4$  to soil on the development of powdery mildew.

#### Effect of air pollution on fungal diseases

There are various reports on the impact of air pollution on fungal plant pathogens and diseases including powdery mildews, both in ambient and glasshouse conditions. The major air pollutants included in the studies are sulphur dioxide ( $SO_2$ ), hydrogen fluoride (HF), ozone ( $O_3$ ) and acid rain.

Sulphur dioxide generally inhibits the growth and development of fungal plant pathogens (Scheffer and Hedgeck, 1955; Skye, 1968). Obligate fungal plant pathogens are reported to be more sensitive to  $SO_2$  than the non-obligate pathogens. This trend has been found both in field and artificial treatment conditions (Weinstein et al., 1975).  $SO_2$  inhibited Puccinia graminis on wheat (Laurence et al., 1979).

Parasitism of bean by Uromyces phaseoli was adversely affected when plants were exposed to  $\text{SO}_2$ , but Alternaria solani was not influenced (Weinstein et al., 1975). Number of lesions caused by Helminthosporium maydis declined by 38% on maize plants exposed to  $\text{SO}_2$  on 8 days before inoculation (0.15 ppm for 14 h/day). If exposed on 8 days before and on the 2 days after inoculations, the decrease in the number of lesions was 13 to 16% only respectively (Laurence et al., 1979). An increase in the number of Schirrhia acicola lesions on needles of Scot pine seedlings was recorded by Weidensaul and Darling (1979) when the seedlings were exposed to 0.20 ppm for 6 h of  $\text{SO}_2$  for 5 days.

Different fungal hyphae show variations in sensitivity to  $\text{SO}_2$  doses. Endophytic hyphae show greater resistance (McCallen and Weedon, 1940). According to Ham (1971) Botrytis and S. acicola were resistant and produced viable conidia when former exposed to 4 ppm  $\text{SO}_2$  for 11 h and latter with 1 ppm of  $\text{SO}_2$  for 4 h. At 90 ppm  $\text{SO}_2$  in nutrient media Aspergillus niger, Alternaria brassicicola and Didymellina macrospora were unaffected and Penicillium showed slightly stimulated growth (Saunders, 1966). In most fungi spore germination was decreased with an increase in water content

(Couey, 1965; Couey and Uota, 1961). At high doses of  $\text{SO}_2$  exposure fungal spores showed great resistance (Couey and Uota, 1961; Hibben, 1966). Puccinia striiformis showed sensitivity to  $\text{SO}_2$  (Sharp, 1967).

The obligate fungal pathogens show more sensitivity to  $\text{O}_3$  than facultative fungal pathogens. Rusts show indirect effect of  $\text{O}_3$ , while powdery mildews show direct effect of  $\text{O}_3$  at the time of conidial formation, germination and penetration. Heagle (1973) observed that mature spores were resistant to  $\text{O}_3$  in ambient conditions. The negative and positive response of  $\text{O}_3$  had been observed on an obligate fungal pathogen Uromyces phaseoli (Rush and Runeckles, 1973).  $\text{O}_3$  produced two times more lesions of facultative fungal pathogen Botrytis on onion plants (Wukasch and Hofstra, 1976, 1977). The time of exposure, stage of fungal development and concentration of  $\text{O}_3$  may affect the response of  $\text{O}_3$  to Helminthosporium maydis on maize leaves (Heagle, 1977).  $\text{O}_3$  injured pine trees died due to infection by Heterobasidion annosum (James et al., 1980). Heagle (1970) found that uredinia of Puccinia coronatum rust of oat were smaller in size when exposed to 0.10 ppm of  $\text{O}_3$  for 6 h 10 days after inoculation. The sporulation of wheat stem rust Puccinia

graminis was inhibited by  $O_3$  due to injured host mesophyll cells and decreased hyphal growth (Heagle and Key, 1973). In culture media the colony growth of fungus, development of aerial hyphae and sporulation were more sensitive to low doses than high doses of  $O_3$  (Ingram and Haines, 1949; Hibben and Stotzky, 1969). According to Hibben and Stotzky (1969) the dry spores of most of the fungi were more resistant than wet spores.

Impact of fluorides on fungal pathogens has been examined by several workers. Treshow (1965) reported that Verticillium albo-atrum, Helminthosporium sativum and Pythium debaryanum in culture media were more sensitive to fluoride than Botrytis cinerea and Colletotrichum spp. On exposure to HF (hydrogen fluoride) parasitism of fungal pathogen was inhibited. The number and growth of the pustules of uredia of Uromyces phaseoli on bean plants leaves was inhibited, when exposed before or after inoculation (Mc Cune et al., 1973). Pre-inoculation exposure of tomato plant to HF is reported to cause decrease in development of Alternaria solani, but no effect on disease development (Mc Cune et al., 1973).

The aeciospores of Cronartium fusiforme on willow oak showed decrease with simulated acid rain. When acidified rain with  $H_2SO_4$  acid at pH 3.2 was applied on each of 14 days before and after inoculation, the number of infections and telia were decreased (Heagle, 1982). Shriner (1978) found an increase in Helminthosporium maydis lesions on maize leaves, when conidia were incubated in water at pH 3.5 before inoculation.

The total number of fungi associated with potato leaves were more in polluted area which indicated that cement dust was not inhibitory to fungi. The maximum number of Penicillium javanicum isolates was found in cement dust areas, while Alternaria solani was less in the dust polluted area (Rai and Pathak, 1981).

#### Powdery mildews

Mostly powdery mildews are exposed to the ambient air during parasitism on their hosts. Various stages of pathogenesis of powdery mildew are affected by any change in atmospheric air e.g. inhibition of conidial germination. Germination of conidia is the first phase of pathogenesis and conidia are the means of secondary spread of the disease. A few records show the impact

of air pollutants on conidial germination of powdery mildew fungi and disease development. Such observations had been made both in ambient and artificial exposure conditions. Microsphaera alni near a paper mill on oak was found to be absent (Kock, 1935). Hibben and Walker (1966) reported a least infection of M. alni on lilac grown in polluted air of New York city and other urban areas than lilac in rural areas. On exposure of leaf-tissue of bean to 300 ppm of fluoride, generally decreased the foliar infection and invasion by Erysiphe polygoni (Treshow, 1965). There was decrease in percentage penetration of E. graminis in barley leaves i.e. 84, 84 and 75% of the controls by 6 h exposure to 0.05, 0.10 or 0.15 ppm of  $O_3$ , but there was no effect on percentage conidial germination and appressorium formation (Heagle and Strickland, 1972). But Schuette (1971) had noted that appressoria formation in E. graminis was slowed by 1 ppm of  $O_3$  during first 8 h of incubation although the germination was not affected. Penetration phase decreased significantly after exposure to 0.25 ppm of  $O_3$  (8-12 h after incubation began), but infection began normally. Hibben and Taylor (1975) observed a reduction in conidial germination, hyphal development and penetration of germ tube, when



was  
Microsphaera alni exposed to .04 ppm SO<sub>2</sub> for 24 h to 72 h daily, while resistant when exposed to 1.0 or 0.25 ppm of O<sub>3</sub> for 6 and 72 h respectively. The number of E. graminis pustule per leaf area of winter wheat was found to decrease when nitrogen was used as fertilizers (Daamen, 1988). There was reduction in incidence and intensity of powdery mildew caused by Sphaerotheca fuliginea on cucurbits like Cucumis sativus, Cucurbita moschata, Cucumis melo and Cucumis melo var. utilissimus grown within 2 km from Ceramic and Pottery Industrial Units, Khurja, where HF is present in higher concentration (Khan et al. 1988 ). The effect of SO<sub>2</sub> in a concentration of 0.1 ppm and 0.2 ppm for different time intervals i.e. (3, 6, 9, 12, 18 h) has been examined on the germination of conidia of some powdery mildew i.e. S. fuliginea on Lagenaria siceraria, E. cichoracearum on Coccinia grandis, S. cassiae on Cassia occidentalis, E. trifolii on Trigonella foneum-graceum, E. pisi on Pisum sativum, E. polygoni on Chenopodium ambrosoides, Microsphaera alphitoides f. sp. zizyphi on Zizyphus jujuba and Phyllactinia dalbergiae on Dalbergia sissoo (Khan and Kulshreshtha, 1991). The germination of conidia of these powdery mildews was observed to be reduced as compared to

controls at both the concentrations of  $\text{SO}_2$ . With increase in duration of exposure a corresponding decrease in germination occurred. Greatest inhibition occurred at the 0.2 ppm concentration with an exposure period of 12 h.

### Root-nodule bacteria

Symbiotic nitrogen fixation by root-nodule bacteria in leguminous crops is a very significant natural biological process. Two genera Rhizobium and Bradyrhizobium are involved in this symbiotic associations. The genus Rhizobium belongs to the family Rhizobiaceae which includes nodule forming bacteria. Several species like R. leguminosarum, R. phaseoli, R. trifolii, R. meliloti and R. lupini etc. comprise the genus. They are fast growing bacteria with sub-polar flagella, having strong affinity for nodule formation towards Lotus and Lupinus. They include the fast-growing strains, nodulating Cicer, Sesbania, Leucaena, Mimosa and Lablab, while the genus Bradyrhizobium has one species i.e. B. japonicum. They are slow growing bacteria with polar or sub-polar flagella forming nodules on soybean, Lotus uliginosus and Vigna. They

include those slow growing strains that form the nodules on Cicer, Sesbania, Leucaena, Mimosa, Lablab and Acacia (Buchanan and Gibbons, 1974). They are rod-shaped of short to medium size, gram negative bacteria. Usually Rhizobium spp. live freely in soil and in the root region of both leguminous and non-leguminous plants. They form a symbiotic relationship with leguminous plants by causing infection to the roots and forming nodules on them. As root nodule become older after a period of nitrogen fixation, decay of tissue occurs, liberating a motile forms of Rhizobium into the soil, thus providing a source of inoculum for the succeeding crop of a given species of the legume (Rao, 1972, 1975). The Rhizobium after penetrating through root hair, form the nodule in the upper cortical regions. The core of mature nodule constitutes the bacteroid zone surrounded by several layers of cortical cells; they show a direct positive relationship with nitrogen fixation. The effective nodules are generally large and pink in colour due to leghaemoglobin (Bergersen and Briggs, 1958).

The nodulated legume system depends not only on the available mineral nitrogen in the soil, but a close relation-

ship is established between host symbiont and the environment. The effectiveness and efficiency of the Rhizobium-legume symbiosis are dependent on this relationship.

There are factors that limit the bacteria ability to compete, their capacity to survive and hydrogenase activity. The photosynthetic rates and nutrient uptake of the host is also limited by various factors. Environmental factors include the P, K, Ca, N, micronutrient and moisture.

Nitrogen fixation was directly and indirectly influenced by the effects of soil acidity on the bacteria and on the host Rhizobium species and strains vary in their tolerance to soil pH. Among tropical and temperate legumes, both tolerant and intolerant species are found and they have different responses to lime (Munns, 1976, Munns et al., 1977).

The effect of soil acidity and pH are related factors on soybean nodulation and  $N_2$  fixation (Freire, 1976). Calcium is required in adequate amount for development of well defined nodulation growth and activity of Rhizobium. Soil acidity and availability of nutrient (such as macro- and micronutrients as Ca, P, Mn, Al, B, Mo and Cu) uptake to plants are correlated to each other. Lime applications correct

Al and Mn and the availability of many other elements in soil.

According to Graham and Halliday (1976), in tropical and sub-tropical areas soil temperature was a major limiting factor for beans. Rhizobium phaseoli can grow at limiting pH 4.0 to 4.4 in liquid media. Paulino et al. (1987) observed a decrease in the number of nodule formation and acetylene reduction of pea plants infected with R. leguminosarum at low pH 5.2.

Nodule formation by R. japonicum on soybean, growth both in broth and soil were affected by temperature. The competitiveness of all inoculum was increased, when temperature was raised from 20 to 35°C. Kluson et al. (1986) reported that vegetative growth of soybeans and nodulation were minimum at 20°C and optimum at 30°C.

When toxic levels of Al and/or Mn are present in the soil, the limiting amount of P fertilizer was required for promoting adequate nodulation. Keyser and Munns (1979) studied the direct effect of Al and Mn toxicities on rhizobia. They reported that in acid soils Mn toxicity and Ca deficiency was less important factor than Al toxicity and acidity, which

limit the rhizobial growth. Potassium, sulphur and micro-nutrients in soil played minor roles as limiting factors at least in areas of low productivity agriculture (Andrew, 1976; Freire, 1976; Franco, 1976). Nitrogenase activity was specifically affected by Al ions and this effect was primarily responsible for the reduction in plant growth (Paulino, 1987).

Aeration was found to be the limiting factor for nodulation of cowpea, soybeans and native legumes during wet periods in black earth soil (Daitloff, 1967). Geopfert and Freire (1973) observed that in a sieved soil of particle size i.e. 0.8 to 2.0 mm, dry matter of Phaseolus vulgaris and nodulation ~~were~~ increased significantly.

According to Franco (1976) more P was required by plants that were dependent on  $N_2$  than the plants using mineral nitrogen.  $N_2$  fixation and legume production was limited by phosphorus deficiency. Phosphorus plays a vital role in energy transfer and large quantity of energy was required for  $N_2$  reduction to  $NH_3$ . The concentrations of P and N in plant tissues increased by addition of P to the soil. This relationship has been shown for tropical pasture legumes (Andrew, 1976). By addition of P, N-concentration

was increased in pasture legumes (Andrew, 1976). Geopfert (1971) reported that grain yield of soybean, P availability in the soil and nodule weight of soybeans had a close relationship.

Becana and Janet (1989) showed a significant reduction in the total content of Lb. in all legumes i.e. Glycine maxima, Pisum sativum, Trifolium repens, Vigna radiata and Vigna anguiculata except in Lupinus luteus, when all the nodules of each host were treated by  $\text{NO}_3^-$ .

Five fungicides (Dithane M45, thiram, blitox, captan emission) and four insecticides (thionel, malathion, aldrin and BHC 50) at different concentrations, were tested for their effect on different Rhizobium spp. Most of the chemicals was non-inhibitory to Rhizobium at lower concentrations i.e. 10 to 100 ppm. At higher concentration the response of various Rhizobium spp. to these chemicals was different (Poi and Ghosh, 1986).

#### Effect of air-pollution on nodules and nodulation

There are several reports on the impacts of air pollution on nodules and nodulation of the host plant by root nodule bacteria. The major air pollutants are  $\text{SO}_2$ ,  $\text{O}_3$ ,

acid rain and fly ash.

In greenhouse or controlled environment chambers 1 or 2 acute  $O_3$  exposures to leguminous plant, reduced the Rhizobium nodulation, nitrogen fixation, and/or leghaemoglobin content (Blum and Heck, 1980; Blum and Tinney, 1977). Peinert and Weber (1980) pointed out that exposures of soybean plants to 0.25 ppm  $O_3$  for 4 h per day, 3 days per week, for 11 weeks, in greenhouse, reduced the number of Rhizobium nodules per plant and nodule weight per plant by 46 and 41% respectively.

At pH 3.2 sulphuric acid reduced the Rhizobium nodulation of kidney beans grown in a greenhouse or field plots and of soybeans grown in greenhouse (Shriner, 1974; Shriner et al., 1981; Waldron, 1978). The soybeans nodulation grown was not reduced by rain of pH 3.5 grown in the field plots (Mc Gruit, 1976). Waldron (1978) separated the effects of  $H^+$  and  $SO_4^-$  ions on nodulation by 'rain' or soil drench application of sulphuric acid (pH 3.2) or sodium sulphate (5.7). He observed that  $SO_4^-$  ions caused a slight reduction in nodulation when applied as 'rain' while  $H^+$  ions caused the major inhibition in nodulation. The inhibition of nodulation also occurred due to substance which was leached by rain from the foilage (Shriner, 1974; Waldron, 1978).



Reduction in the number and dry weight of the bacterial nodules occurred, on exposure of soybean plants to  $O_3$  (Reinert and Weber, 1980). Klarrer et al. (1984) pointed out that nodule number was significantly reduced, while there was no effect on individual nodule size, when soybean plants were exposed to  $SO_2$  and  $NO_2$  alone or in mixture. This reduction in nodule number was due to reduction in N-fixation.

Acidity of the medium was correlated with the growth inhibition of chickpea and lentil strains of Rhizobium. At pH 6 growth of lentil strains was slightly better, while the growth of chickpea strain were significantly unaffected. The chickpea strains of Rhizobium was apparently more sensitive to acidity than lentil strain. At pH 3.2 total growth inhibition of chickpea strain occurred, while lentil strain growth was inhibited at pH 2.5. Total number of nodules and the functional nodules decreased significantly at both the pH of rains in chickpea and lentil strain (Singh, 1989). The growth of chickpea and lentil strain of Rhizobium was suppressed, when exposed to  $SO_2$  at concentration 0.1 ppm and 0.2 ppm and  $O_3$  at concentration alone or in combination. Inhibitory effect were more at the higher

concentrations. Chickpea strain had a greater sensitivity to  $\text{SO}_2$  exposures and lesser sensitivity to  $\text{O}_3$  exposure than lentil strain. There was greater reductions in the growth of chickpea strain than lentil strain when exposed to a mixture of  $\text{SO}_2$  and  $\text{O}_3$  at both concentrations. Nodulation was also observed to be reduced in number/root system (Singh, 1989).

In artificially amended soil with fly ash at different levels i.e. from 10 to 100%, showed a stepwise decrease in the nodule formation of Rhizobium, with an increase in levels of fly ash of soil and completely suppressed at 70% and 80% levels of fly ash in chickpea and lentil strains respectively (Singh, 1989). Root nodulation (number of nodules/root system) was significantly suppressed in fly ash amended soil, in both of chickpea and lentil strain.

## MATERIALS AND METHODS

In the proposed study, effect of gaseous air pollutants ( $\text{SO}_2$  and  $\text{O}_3$ ) on the crop performance and development of powdery mildew fungi (Erysiphe spp. and Sphaerotheca spp.) and root nodule bacteria (Rhizobium) on blackgram (Vigna mungo (L.) Hepper.) and pea (Pisum sativum L.) will be studied in pots in glasshouse.

Plants will be exposed to different doses of the air pollutants in exposure chambers. The following will be the pattern of treatments.

### (a) Control sets

$T_1$  - Plant

$T_2$  - Plant + powdery mildew

$T_3$  - Plant + root nodule bacteria

$T_4$  - Plant + root nodule bacteria + powdery mildew

### (b) Exposed sets

$T_5$  - Plant + air pollutant ( $\text{SO}_2/\text{O}_3$ )

$T_6$  - Plant + powdery mildew + air pollutant ( $\text{SO}_2/\text{O}_3$ )

$T_7$  - Plant + root nodule bacteria + air pollutant  
( $\text{SO}_2/\text{O}_3$ )

$T_8$  - Plant + root nodule bacteria + powdery mildew +  
air pollutant ( $\text{SO}_2/\text{O}_3$ ).

Each treatment will be replicated six times and pots will be arranged in randomised block design on glasshouse benches.

At the termination of experiments, the following parameters will be determined for each treatment:

Root and shoot lengths

Fresh and dry weights of shoot

Fresh and dry weights of root

Per cent infected leaf area

Conidial size

Germination of conidia

Detection of air pollution symptoms

Number and weight of nodules, functional and non-functional nodules/root system

Chlorophyll content of leaf

Nitrogen and phosphorus contents of roots and leaves

Protein content of seeds.

#### Exposure System

##### Exposure chamber

Exposure chambers (Standard Appliances, Varanasi)

will be used for the exposure of the test plants or other materials to a mixture of air and the air pollutant involved.

The front of air pollutant exposure chamber has a full sized door and exhaust duct is provided at the top to carry out the air/gaseous mixture. The bottom is double walled and upperside has openings and the lower side is equipped with a special type of blower assembly. A voltage controller regulates the voltage supply to blower which is displayed on the panel meter. The plants in pots will be kept in the exposure chamber and exposed for a desirable length of time.

#### Gas generation

Sulphur dioxide will be generated in a generator which produces  $\text{SO}_2$  gas by the action of sulphuric acid ( $\text{H}_2\text{SO}_4$ ) on sodium sulphite ( $\text{Na}_2\text{SO}_3$ ) under control reaction conditions. The amount of  $\text{Na}_2\text{SO}_3$  and  $\text{H}_2\text{SO}_4$  acid discharged from the reagent bottles mounted over the  $\text{SO}_2$  generator will be determined by collecting the solution dropping through capillary tube in a graduated cylinder for sometime and expressing the rate in ml/min. On the basis of flow rate or solution feeding rate, solutions of sodium sulphite ( $\text{Na}_2\text{SO}_3$ ) and sulphuric acid (10%) will be prepared to produce required amount of  $\text{SO}_2$  gas/min. On complete

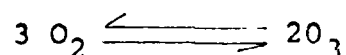
reaction 1M  $\text{Na}_2\text{SO}_3$  produces 1SO<sub>2</sub> or 126 mg  $\text{Na}_2\text{SO}_3$  produces 64 mg SO<sub>2</sub>.



10%  $\text{H}_2\text{SO}_4$  acid solution will be used for all the working solutions of  $\text{Na}_2\text{SO}_3$ .

### Ozone

Ozone will be generated by subjecting dry oxygen to the action of silent electric discharge in an apparatus called Ozoniser.



### Powdery Mildew

Surveys of pea and black gram fields will be conducted in order to collect the inocula of the powdery mildews.

### Identity of causal organism(s)

Samples of pea and black gram apparently infected with powdery mildew will be brought to the laboratory from fields. The samples will be thoroughly examined for infection, with the help of dissecting microscope and magnifying glass. Morphological characteristics of the different structures of anamorph and telomorph, if present,

will be studied microscopically and their dimensions will be measured. Conidia will then be subjected to fibrosin bodies test and germination test in order to study the absence or presence of fibrosin bodies and the morphology of germ tubes and appressorial development. These observations on anamorph characters will help in establishing the identity of the pathogens.

To test the presence of fibrosin bodies in conidia, conidia from all the samples will be treated on clean glass slides with 3% KOH aqueous solution. Conidia will be then examined under the microscope (Kable and Ballantyne, 1963) and presence or absence of well developed fibrosin bodies will be noted.

Conidia from each sample will also be subjected to germination test in order to study the mode of germination and morphology of germ tubes and development of appressoria. For the test, conidia will be gently dusted on clean glass slides. At least 3 such slides from each sample will be then placed on glass rod triangles, kept in petriplates containing moistened cotton at the bottom. The petriplates will be incubated at  $20^{\circ}\text{C}$  ( $\pm 2$ ). At the end of the incubation period (24 h), slides will be examined for

observing the germination of conidia. The morphology of germ tubes will be studied and percentage germination of conidia will be determined by random counting of germinating and non-germinating conidia in a number of microscopic fields from each slide. A set of slides from each sample incubated in the same way as described above will be examined after 48 h for detecting the development of appressoria. The morphology of the appressoria will be studied and noted.

#### Culturing and maintenance of the pathogen

For experimental studies in glasshouse conditions, inocula from selected samples considered necessary, preferably from areas of high disease intensities, will be maintained in glasshouse on young plants of the leguminous crop grown in pots containing autoclaved soil. Plants of p. sativum for Erysiphe pisi and Vigna mungo for Sphaerotheca fuliginea will also be used for maintenance whenever required.

#### Powdery mildew inoculation

Inoculations will be done by dry dusting of conidia or appressing infected areas of the leaves on the leaf



surface of the seedlings. Subsequent inoculations when necessary will be done to maintain the inocula for desired periods.

#### Root Nodule Bacteria

To assess the impact on root-nodulation, number of total, functional and non-functional root nodules per plant present in the samples collected from the glasshouse experiments will be counted. The pinkish healthy nodules will be taken as functional and others as non-functional.

#### Isolation of Rhizobium

Rhizobium spp. will be isolated from pea and black gram collected from fields. Isolation will be made separately. After washing the root system of the plants in running water, a well formed healthy pinkish nodule on the tap root will be carefully cut out with a portion of root attached to the nodule. The nodule will be surface sterilized for 5 min. in 0.1% mercuric chloride and repeatedly washed with sterile water to remove the chemical. The nodule will then be washed in 70% ethyl alcohol for 3 min. followed by more washing with sterile water (Ash and Allen, 1948). The nodule will be now crushed with sterile

glass rod in a small aliquot of sterile water. This will be diluted for obtaining clear and distinct colonies. Congo red yeast-extract mannitol agar medium (CPYMA) will be used for isolation. The constituents of the medium are as follows:-

Mannitol	10.0 g
Yeast extract	1.0 g
NaCl	0.1 g
$K_2HPO_4$	0.5 g
$MgSO_4 \cdot 7H_2O$	0.2 g
Agar-agar	20.0 g
Distilled water	1000 ml.
Congo red	2.5 ml. of 1% solution.

1 ml of the dilution will be added to each petriplate containing 15 ml of CRYMA medium. The petriplates will be incubated at  $30^{\circ}C (\pm 2)$  for one week. Distinct white, translucent, glistening, elevated colonies of Rhizobium developing on the media will be picked up and purified by reculturing.

#### Plant testing methods (Rao, 1975)

As variations are found in strains of Rhizobium, it is necessary to determine the ability of a particular isolate to produce nodules on a suitable host legume.

Similarly, it is also essential to know that the nodules possess efficient nitrogen fixing ability. This will be ascertained by growing plants on agar slants containing nitrogen-free medium and inoculating them with the desired isolates. At regular intervals, seedlings will be fixed in 4% formalin and be examined later under a microscope for infection threads in root-hairs and emergence of nodule primordia on roots. Depending on the extent of infection, isolates may be rated for virulence.

In another set, the experiment will be continued for several weeks for obtaining the effectiveness of Rhizobium isolate by examining the dry weight of the plants. The dry weight of plant is proportional to its N-content (Erdman and Means, 1952). During the experiment the moisture content of the culture tube will be checked at the regular intervals and the nutrient solution may be changed when necessary.

#### Nitrogen-free nutrient media (Jensen, 1942)

CaHPO <sub>4</sub>	1.0 g
K <sub>2</sub> HPO <sub>4</sub>	0.2 g
NaCl	0.2 g
MgSO <sub>4</sub> 7H <sub>2</sub> O	0.2 g
FeCl <sub>3</sub>	0.1 g
Agar-agar	8.0 g
Distilled water	1000 ml
pH	6.8

### Pure culture of Rhizobium

Normally yeast extract mannitol agar (YMA) is used for pure culturing of Rhizobium (Fred et al., 1932) Rothamsted collection of Rhizobium (RCR) have modified it slightly.

The compositions of YMA is as:

K <sub>2</sub> HPO <sub>4</sub>	0.5 g
Mannitol	10.0 g
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.2 g
NaCl	0.1 g
Yeast extract	0.4 g
Agar-agar	15.0 g
Distilled water	1000 ml
pH	6.8-7.0

The medium will be autoclaved at 15 lb. p.s.i. (121°C) for 20 minutes. Then media will be poured in sterile petriplates. After solidification of medium, the tested Rhizobium isolate will be inoculated in the plates in aseptic conditions at laminar flow bench. After inoculation, the petriplates will be kept at 30°C (± 2) in an incubator. The colonies will grow in about seven days. The culture can also be done in culture tubes.

### Soil-based culture

For inoculating the legumes in pots, the soil based culture of Rhizobium will be used for seed dressing prior to sowing.

For culturing Rhizobium in soil, the soil and compost in the ratio 1 : 1 will be used. 1 kg of soil-compost mixture will be autoclaved and the pH will be maintained at 7 by mixing 10 g of  $\text{CaCO}_3$ . After that 10 gm sugar (commercial) and 0.5 gm  $\text{K}_2\text{HPO}_4$  will be added in soil-compost mixture. Then pure culture of Rhizobium grown on YMA will be mixed thoroughly. This mixture of Rhizobium and soil-compost will be used for inoculating the seeds of particular pulse crop before sowing.

### Rhizobium inoculation

Rhizobium inoculation will be done prior to seed sowing. For inoculation soil-based culture of Rhizobium will be used. Commercial sugar and water will be added in the soil based culture with thorough mixing. The pulse seeds will be treated with this mixture followed by the drying in shade for about half an hour before sowing.

### Exposure and Doses

3-4-week-old seedlings of the test plants (pea, black gram) will be exposed to  $\text{SO}_2$  and/or  $\text{O}_3$  at every alternate day for 3 h. This procedure will be continued for 75 days. The concentration of gases used for exposure will be 0.1 and 0.2 ppm.

### Plant Growth

For assessing the effect of the air pollutants on plant growth of pea and black gram, samples from the pot experiments will be collected in polythene bags and labelled. In laboratory the root system of the plants will be thoroughly washed to remove the soil particles. Root and shoot lengths, fresh and dry weights of root and shoot will be determined for each sample by standard methods.

### Air Pollution Symptoms

Plants collected from the glasshouse experiments will be invariably examined closely in the laboratory for detecting symptoms on plants. Symptoms will be characterized and noted. Symptoms will also be matched with the symptoms given in "Recognition of air pollution injury to

vegetation: a pictorial atlas (Eds. S. Jacobson and A.C. Hill), Air Pollution Control Association, Pittsburgh, Pennsylvania, 1970.

#### Parameters

##### Measurement of per cent infected leaf area

Powdery mildew infected leaves will be detached acropetally from the plants and placed on a sheet of white paper. A tracing paper will be placed over the leaves to draw the outline of entire leaf margin and infected portion of the leaves with the help of a pencil. The total leaf area ( $\text{cm}^2$ ) and infected area ( $\text{cm}^2$ ) will be then recorded with planimeter

$$\frac{\text{Percent infected leaf area}}{\text{leaf area}} = \frac{\text{Infected leaf area}}{\text{Total leaf area}} \times 100$$

##### Conidial size

Conidial size of the powdery mildew (length x breadth) measured by will be ~~by~~ calibrating ocular micrometer placed in eye-piece. Measurements will be made under low or high power objectives of the microscope as found necessary.

##### Germination of conidia

Germination of conidia from the experimental samples

will be determined by the same method as described earlier under the "identity of the pathogens". Percentage germination will be calculated.

#### Plant Analysis

Analysis of plant samples from glasshouse experiments will be done for estimating chlorophyll, nitrogen (N), phosphorus (P) and protein contents.

#### Estimation of chlorophyll contents

Chlorophyll contents of leaves of the plant samples collected from glasshouse experiments will be estimated. 1 g of interveinal region of the leaves will be ground in 40 ml 80% acetone with the help of mortar and pestle. The suspension will be decanted in buchner funnel having two Whatman paper no. 1. Then filtration will be done with the help of suction pump. The residue will be ground thrice adding with 30, 20 and 10 ml of acetone respectively. The suspension will be decanted in buchner funnel and filtered in vaccum. At last mortar and pestle will be rinsed with 80% acetone, transferred in buchner funnel and filtered in vaccum. The filtrate will be transferred in 100 ml volumetric flask and the volume will be made upto capacity. The transmittance will be read at 645, 663 and



635 nm at spectrophotometer. The chlorophyll a, b and total chlorophyll will be calculated accordingly by using optical density (O.D.) i.e. by using % transmittance (Mackinney, 1941).

Chl. a. in fresh tissue =  $12.7 \text{ (O.D. 663)} - 2.69$

$$(\text{O.D. x 645}) \times \frac{V}{1000 \times W}$$

chl. b. in fresh tissue =  $22.9 \text{ (O.D. 645)} - 4.68$

$$(\text{O.D. 663}) \times \frac{V}{1000 \times W}$$

Total Chl. in fresh tissue =

$$20.2 \text{ (O.D. 645)} + 8.02 \text{ (O.D. 663)} \times \frac{V}{1000 \times W}$$

#### Estimation of N and P

For estimation of N and P root and leaf samples will be digested as given below

#### Digestion of leaf and root samples

Leaf and root samples from the glasshouse experiments will be digested first according to the following method:

100 mg of oven dried leaf and root powder will be transferred in 50 ml Kjeldhal flask, then 2 ml of chemically pure  $\text{H}_2\text{SO}_4$  will be added and flasks will be heated on kjeldhal assembly for about 2 h, till the dense fumes have

given-off and the contents have turned black. Then 0.5 ml of pure 30%  $H_2O_2$  will be added after 15 min. of cooling. Now heating will be done again till the colour is changed into light yellow. It will be heated again for half an hour and after which flask will be cooled for 10 min. for getting extract clear. Then 3-4 drops of 30%  $H_2O_2$  will be added dropwise followed by heating for 15 min. After that digested material will be transferred in 100 ml volumetric flask with 3-4 washing and will be used for estimating N and P etc. present in the leaf and roots (Linder, 1944; Lundegardh, 1951).

### Nitrogen

Prior to estimating the N content present in the digested material of leaf, standard curve will be drawn by the following procedure.

0.236 g of ammonium sulphate will be dissolved in 100 ml of solution, then 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1.0 ml solution will be poured in 10 test tubes respectively. The volume will then be made upto 5 ml in each test tube by adding distilled water. A control will also be run side by side. After that 0.5 ml Nessler's reagent will be added followed by 5 ml of distilled water.

The percentage transmittance will be read at 525 nm from spectrophotometer on developing yellow orange colour after half an hour. Then a curve will be drawn on graph between concentration and O.D.

### Estimation

10 ml of aliquot (digested leaf and root material) will be taken in 100 ml volumetric flask and 2 ml, 2.5 N NaOH will be added to neutralise the excess amount of acid present. 1 ml of 10% sodium silicate will also be added to prevent turbidity. Then volume will be made upto capacity. 5 ml of aliquot will be taken in 3 test tubes followed by addition of 0.5 ml of Nessler's reagent with shaking, then 10 ml volume will be made by distilled water. After waiting for 5 min. the % transmittance will be read at 525 nm. Then the O.D. will help in reading the concentration from the standard curve (Linder, 1944).

### Phosphorus

At first a standard curve will be prepared. Different concentrations of  $\text{KH}_2\text{PO}_4$  solution ranging from 0.1 to 1 ml will be taken in 10 separate test tubes and the volume of each test tube will be maintained upto 5 ml. Then 1 ml ammonium molybdic acid and 0.4 ml of 1 amino-2-nepthol-4

sulphonic acid in each test tube will be added followed by making the volume upto 10 ml with distilled water. After half an hour % transmittance will be read at 625 nm. Then standard curve will be drawn between concentration and O.D.

### Estimation

5 ml of aliquot (digested leaf and root) will be taken in three test tubes to which 5 ml of distilled water will be added. After that 1 ml of ammonium molybdic acid will be added, with shaking, followed by addition of 0.2 ml. 1-amino-2-nepthol-4-sulphonic acid. The control will also run side by side. Percentage transmittance will be read at 625 nm after half an hour. Concentration will be read from standard graph by using O.D. (Fiske & Row, 1925).

### Protein estimation

The protein contents of the pulses will be estimated by using the method given by Lowry et al., (1951).

Following reagents will be prepared for estimating soluble and insoluble protein contents of pulse seeds

Reagent A - 2.5% sodium carbonate in 0.1 N NaOH in ratio of  
1 : 1.

Reagent B - 0.5%  $\text{CuSO}_4$  in 1% sodium tartrate in ratio of  
1 : 2.

Reagent C - 50 ml reagent A + 1 ml reagent B.

(Alkaline  $\text{CuSO}_4$ )

Reagent D - 50 ml of 2% sodium carbonate + 1 ml reagent B.

(Carbonate  $\text{CuSO}_4$  solu.)

Reagent E - Follin's reagent diluted to make 1 N acid.

(Diluted follin's reagent).

### Standard curve

Before actual estimation a standard curve will be prepared by dissolving 40 mg of egg albumin in 0.1N NaOH solution, the volume will be made upto 100 ml from this solution, aliquots of 0.1 ml to 1 ml will be taken in 10 test tubes. Reagent A will now be added to the test tubes. After 10 min 0.5 ml reagent E will be added to the test tubes. The % transmittance will be read at 770 nm and standard curve will be drawn between O.D. and concentrations.

### Soluble proteins

50 mg dry powder of seeds will be ground with 5 ml of double distilled water in mortar and pestle. Then water extract will be decanted in centrifuge tube for centrifugation at 400 rpm for 10 min. Then supernatant will be collected in 50 ml volumetric flask and residue will be retained in centrifuge tube for estimating insoluble proteins.

After making the volume upto 50 ml, 1 ml of water extract will be transferred in a 10 ml test tube followed by addition of 5 ml of reagent C. After mixing, solution will be left as such for 10 minutes. Then 5 ml of reagent E will be added and mixed immediately. The control will be run along with experimental set. Percent transmittance will be read at 660 nm after half an hour. The corresponding protein content will be measured, by using the standard curve.

#### Insoluble proteins

The residue retained in the centrifuge tube will be used for insoluble protein estimation. 5 ml of 5% trichloroacetic acid will be added to the residue with shaking. After half an hour it will be centrifuged at 4000 rpm to 10 min. The supernatant will be discarded. 5 ml of 1N NaOH will be added in the residue with vigorous shaking. After half an hour it will be again centrifuged and supernatant will be collected in 50 ml volumetric flask and volume will be made upto within 1N NaOH.

1 ml of this solution will be taken in test tube with 5 ml of reagent D followed by mixing. After 10 min. 0.5 ml of reagent E will be added with immediate mixing. 1N NaOH

will be used in control. Percent transmittance will be read at 660 nm after 30 min. The protein content will be calculated by using the standard curve.

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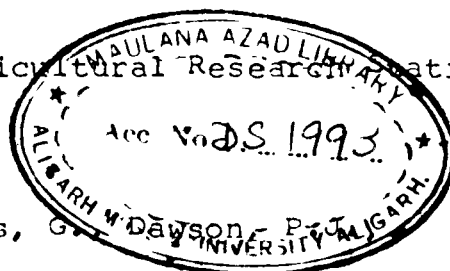
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